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(54) Method and apparatus for continuously separating high molecular weight amphoteric electrolytes by electrophoresis

(57) Apparatus for continuously separating by electrophoresis comprises a casing 1, an electrophoretic membrane 2 partitioning inside of the casing, dialytic membranes 3 and 4 partitioning respective chambers 5 to 8 between the electrophoretic membrane and both side walls of the casing, and electrodes 9 and 10 respectively disposed in the chambers 5 and 8 between the dialytic membranes 3 and 4 and the side walls. Buffer liquid is circulated through the chambers 5 and 8 containing the electrodes, and a sample solution containing an amphoteric high molecular weight electrolyte is circulated through chambers 6 and 7 between the electrophoretic membrane and the dialytic members. DC voltage is impressed across the electrodes. According to this invention it is possible to continuously separate a large quantity of sample into different compositions.

FIG. 1

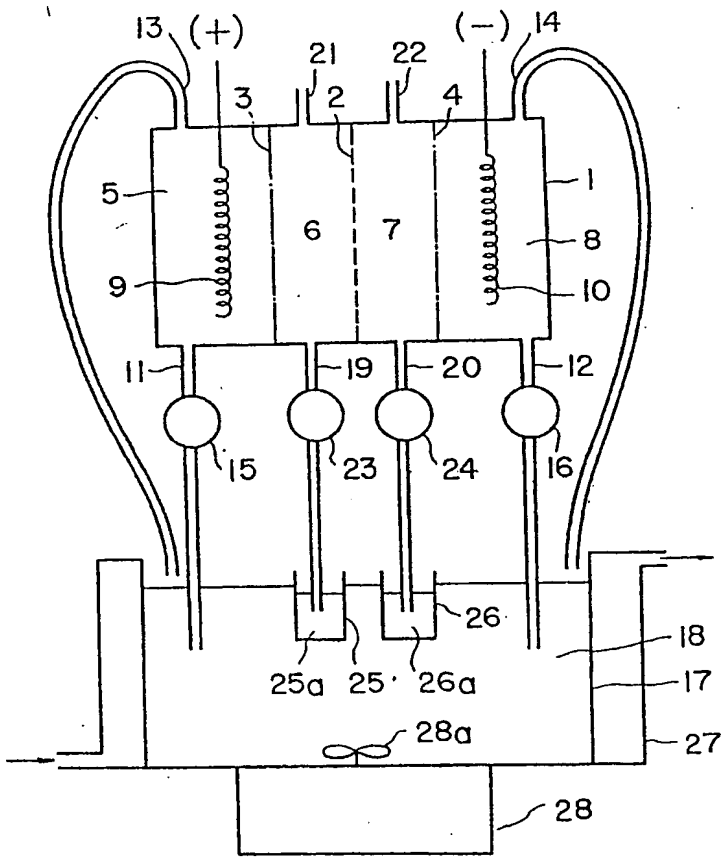

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FIG. 1

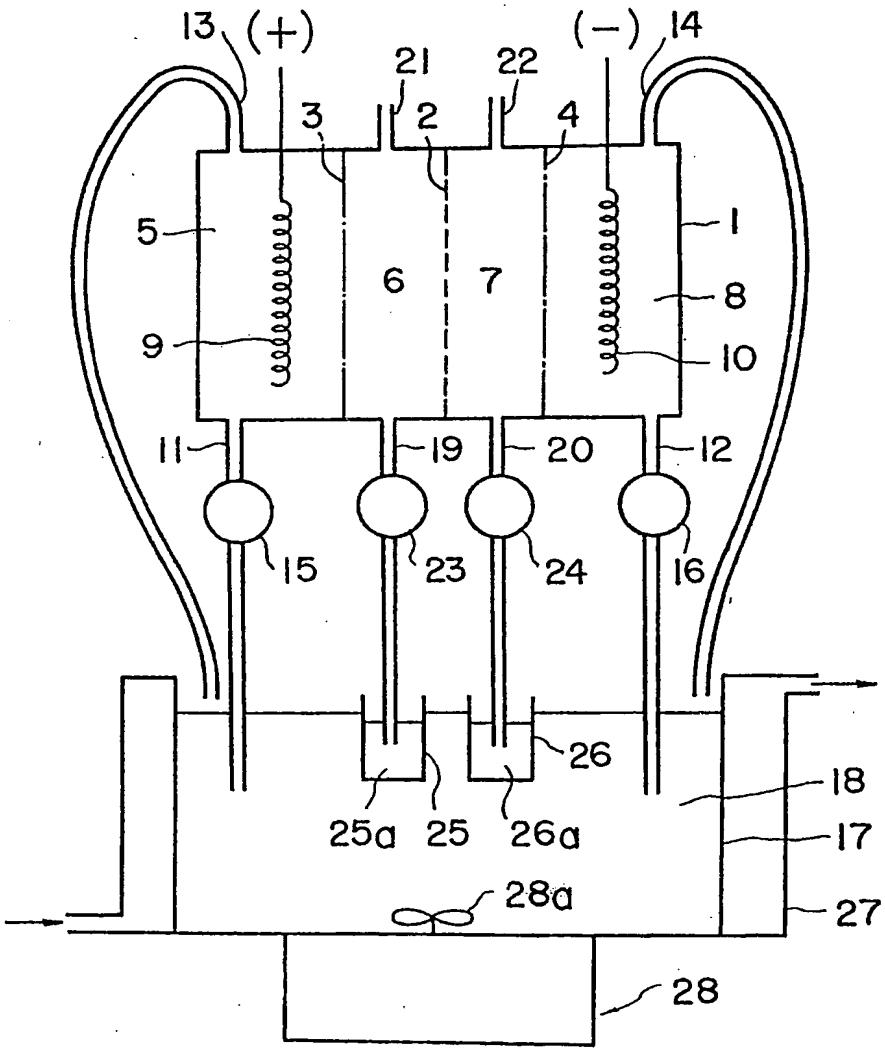
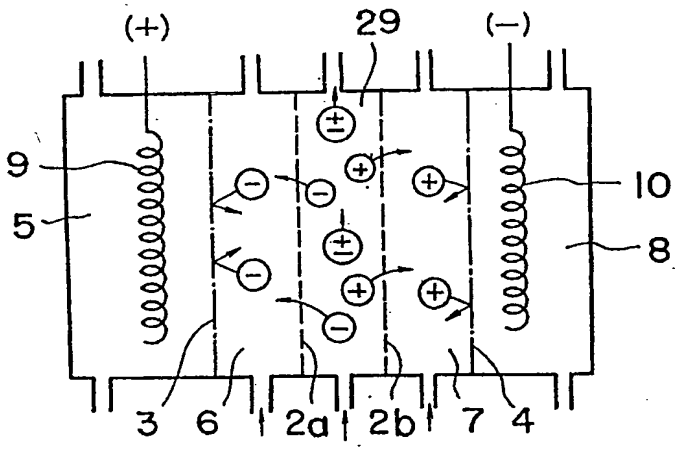


FIG. 2



SPECIFICATION

Method and apparatus for continuously separating high molecular weight amphoteric electrolytes by electrophoresis

Background of the Invention

This invention relates to a method and apparatus for continuously separating a high molecular weight amphoteric electrolyte by electrophoretic effect.

Albumin, a typical example of high molecular weight amphoteric electrolytes has an important substance as an enzyme or as a substance for constituting various portions of a living body so that its quick separation contributes greatly to biochemistry. As a method of separating albumin and other high molecular weight amphoteric electrolyes is known an electrophoretic method. Since according to a commonly used electrophoretic method, a sample of a definite quantity is separated into respective component elements, the quantity that can be separated by one operation is very small, there is a problem at the time of scaling up. Furthermore, an electrophoretic device designed for continuously separating a sample operates to simultaneously separate sample constituent elements into a plurality of fractions so that it is not suitable for use in a preparative scale.

Summary of the Invention

It is an object of this invention to provide a method and apparatus for continuously separating a solution of a mixture of high molecular weight amphoteric electrolytes into liquids having different compositions.

According to one aspect of this invention, there is provided a method of continuously separting an amphoteric high molecular weight electrolyte by electrophoresis comprising the steps of adjusting pH of a sample solution containing the amphoteric high molecular weight electrolyte according to an equipotential point of the electrolyte, contacting the sample solution in a flowable state with a buffer liquid having the same pH as the sample solution and also in a flowable state via a dialytic membrane, applying electric voltage to the sample solution from the side of the buffer liquid to cause the amphoteric high molecular weight electrolyte in the sample liquid to migrate electrically thereby separating the amphoteric high molecular weight electrolyte.

According to another aspect of this invention, there is provided apparatus for continuously separating by electrophoresis comprising a casing, an electrophoretic membrane partitioning inside of the casing, dialytic membranes partitioning respective chambers between the electrophoretic membrane and both side walls of the casing, electrodes respectively disposed in the chambers between the dialytic membranes and the side walls, means for circulating buffer liquid contained in a tank through the chambers containing the electrodes, means for circulating a sample solution containing an amphoteric high molecular weight electrolyte and contained in another tank through chambers between the electrophoretic membrane and the dialytic members, and means of applying DC voltage across the electrodes.

Brief Description of the Drawings

In the accompanying drawings:

Fig. 1 is a diagrammatic representation of one embodiment of this invention, and

Fig. 2 is a diagrammatic representation of a modified embodiment of this invention.

Description of the Preferred Embodiments

Before describing preferred embodiments of this invention, the basic principle of this invention will firstly be described.

When an aqueous solution of an amphoteric electrolyte is subjected to an electrophoretic effect, molecules of the electrolyte migrate in the aqueous solution according to their mobilities, and their speeds and directions of movement are governed by the mass and electric charge of the electrolyte. In the case of the amphoteric electrolyte, the electric charge varies with pH and at a pH corresponding to an equipotential point, the sum of the positive and negative charges of the amphoteric electrolyte molecules becomes zero and becomes positive or negative at a pH higher or lower than the pH corresponding to the equipotential point. Consequently, for specific amphoteric electrolyte molecules it is possible to make positive or negative the charge in a solution by suitably selecting the pH of the solution. This invention is based on a unique utilization of this fact, and according to this invention the pH of a solution containing two types of amphoteric electrolyte molecules is selected to a suitable value so as to make opposite the directions of migrations of the molecules when subjected to electrophoretic effect, thus separating the molecules.

In carrying out the method of this invention, a solution prepared to satisfy the condition just mentioned is charged into a vessel partitioned by an electrophoretic membrane which permits free passage of ions of the electrolyte but prevents movement of the solution, for example a membrane made of carrier gel utilized in the electrophoresis. Electrodes are disposed on the opposite sides of the membrane to produce electrophoresis so as to move positive ions to the positive electrode and move negative ions to the positive electrode. Consequently, positive molecules on the positive electrode side migrate to the negative electrode side through the membrane, while negative molecules on the negative electrode side to the positive electrode. In the absence of electrolysis at the electrode surfaces, both amphoteric electrolytes would accumulate in chambers or compartments on the opposite sides of the membrane.

In order to satisfactorily separate the molecules, it is essential to prevent the molecules of both electrolytes from contacting the electrodes and electrolized, and to limit the

variation in pH of the solution caused by electrophoresis to a negligibly small value. This is only possible by surrounding electrodes with membranes that passes ions but presents passage of the amphoteric electrolyte, by filling buffer liquid into the spaces surrounded by the membranes so as to prevent the amphoteric electrolyte molecules from contacting the electrodes and by constantly exchanging the buffer liquid so as to maintain the pH thereof at a constant value.

For example, such dialytic membranes made of cellophane may be used to surround the electrodes and such higher molecular weight amphoteric electrolyte as albumin may be used as a sample to be separated. However, it is essential that both the dialytic membrane and electrophoretic membrane do not undergo electrophoresis at the time of current passing or can limit the electrophoresis to a negligibly small value. For this reason, filter paper and cellulose acetate films are not suitable for the dialytic membranes because their permeability to the solution is high and because their electric permeability is also high cellophane is also not suitable for a dialytic membrane because it accompanies electric permeability and manifests permeability to low molecular weight albumin. For this reason, the membrane should be made of other materials.

Where apparatus that can fulfill these conditions is used, it becomes possible to separate a solution containing two or more high molecular weight amphoteric electrolytes having different equipotential points into two or more solutions containing different compositions according to the difference in the equipotential points. When such a separation is repeated by adjusting pH, it is possible to separate the solution into much more fractions.

To isolate a specific substance out of a mixed sample containing a member of amphoteric electrolytes, two pH values are set on the opposite side of the equipotential point of the specific substance, and the pH values of the sample solution and the buffer liquid are adjusted to be equal to one of the pH values. Then an isolation operation is made to obtain a fraction containing the specific substance. Then the value of pH is adjusted to be equal to the other pH and thereafter isolation is made to obtain a fraction containing only the object composition.

The actual separation operation is extremely simple. Thus, a sample is dissolved in a buffer liquid, its pH is adjusted to a predetermined value, the buffer liquid filled in two separation compartments on the opposite sides of an electrophoretic membrane and current is passed between positive and negative electrodes contained in electrode chambers partitioned from the separation chambers by dialytic membranes while circulating a buffer liquid having the same pH as the sample liquid through the electrode chambers.

Although at the time of current flow, Joule heat generates, acid and base generated on the surfaces of both electrodes as a result of electrolysis vary pH of the buffer liquid to render difficult to separate. Such variations in the separation operation can be prevented or alleviated by increasing the circulating speed of the buffer liquid and cooling the same to a constant value. Even when the values of pH of the liquids returned from the positive and negative electrode chambers differ slightly, the original value of pH can be resumed by admixing both liquids. Accordingly, it is not necessary to prepare a large quantity of the buffer liquid for an operation over a long period.

This method of separation has a novel advantage in that the sample concentration of the separated solution is higher than that of the sample solution. When sample liquids having the same composition are poured into two separation chambers, a portion of the solute in the sample solution migrates from one chamber to the other, thus increasing the solute in the latter. Separation of a substance generally accompanies decrease in the concentration so that concentration after separation is often required. The method of this invention is an exceptional case. Furthermore, according to this method, it is possible to greatly increase the concentration by merely adjusting the supply speed of the sample liquid.

Apparatus suitable for carrying out the method of this invention will now be described.

As shown in Fig. 1, the casing 1 of the apparatus is made of an electric insulating material and the inside of the casing is partitioned into an electrode chamber 5, separation chambers 6 and 7 and an electrode chamber 8 by an electrophoretic membrane 2 and two dialytic membranes 3 and 4. Electrodes 9 and 10 are disposed in the electrode chambers 5 and 8 respectively. Electrode chambers 5 and 8 are provided with buffer liquid inlet pipes 11 and 12 at their bottoms and buffer liquid discharge pipes 13 and 14 at their upper sides. Feed pumps 15 and 16 are included in the inlet pipes 11 and 12 having lower ends dipped in buffer liquid 18 contained in a tank 17. The lower ends of the discharge pipes 13 and 14 open into the tank 17.

The bottoms of the separation chambers 6 and 7 are connected to sample liquid inlet pipes 19 and 20 and their upper sides are connected to discharge pipes 19 and 20. Feed pumps 23 and 24 are included in the inlet pipes 23 and 24, the lower ends thereof being dipped in sample liquids 25a and 26a contained in independent tanks 25 and 26. Although not shown, the discharge pipes 21 and 22 are connected to tanks 25 and 26 respectively.

A cooling jacket 27 is provided to surround the buffer liquid tank 17 and a magnetic stirrer 28 with blades 28a, for example, is disposed at the bottom of the tank 17.

The electrophoretic membrane 2 and the dialytic membranes 3 and 4 substantially prevent permeation of the liquid, thus manifesting ion conductivity for the electrophoresis. The dialytic

membranes 3 and 4, however, do not permeate ions of high molecular weight substance.

Sample liquids 25a and 26a with their solute concentration and pH adjusted are poured into the sample liquid tanks 25 and 26, while the buffer liquid 18 with its pH adjusted to be the same as that of the sample liquid is supplied to the buffer liquid tank 17. Thereafter, pumps 15, 16, 23 and 24 are started to circulate the buffer liquid through the electrode chambers 5 and 8 and the sample liquid through the separation chambers 6 and 7. (+) and(—) voltages are applied to electrodes 9 and 10 from a source of direct current, not shown. Then the positive ions begin to migrate toward the negative electrode while the negative ions toward the positive electrode respectively through the electrophoretic membrane 2, thus passing electric current. The two dialytic membranes 3 and 4 permeate ordinary ions but not high molecular weight ions, the range of migration of the high molecular weight electrolyte ions in the sample liquid in the separation chambers 6 and 7 being limited within the separation chambers 6 and 7, with the result that the positive ions of the high molecular weight electrolyte concentrate in the separation chamber 7, while the negative ions in the separation chamber 6.

Where the high molecular weight electrolyte comprises albumin or other high molecular weight amphoteric electrolyte, since the high molecular weight amphoteric electrolyte having a higher equipotential point than the pH utilized herein will have a positive charge, such electrolyte will concentrate in the separation chamber 7, whereas those having a lower equipotential point has a negative charge so that it will concentrate in the separation chamber 6. Accordingly, the sample liquid is separated into two types of liquids having different compositions.

Where the volumes of the separation chambers are made equal, the concentrations of the solutes contained in respective fractions would become twice of the initial concentration when the separation is completed; but when the sample liquid is divided into fractions of different volumes, the solute collected in the sample liquid tank having smaller volume would have higher concentration, thus effecting more efficient concentration.

Current not only produces Joule heat throughout the entire region of the casing, but also results in the electrolysis of the solution on the electrode surfaces, thereby varying the pH of the buffer liquid. However, the Joule heat thus generated is removed by a large quantity of the buffer liquid circulated through the electrode chambers 5 and 8 by feed pumps 15 and 16, thereby preventing temperature rise and denature of the sample. Where the material to be electrolyzed comprises water, any variation in pH occurring at the same time can be decreased to a minimum by increasing the quantity of the buffer liquid. By returning the buffer liquid discharged through the discharge pipes 13 and 14 back to the buffer liquid tank 17 and by stirring with the stirrer 28, the pH value can be returned to the original value so that it is not necessary to provide any device for maintaining the pH at a definite value for the continuous operation. During running, cooling water is passed through the jacket 27 to prevent temperature rise of the buffer liquid 18.

It is desirable that the speeds of the pumps 15 and 16 for feeding the buffer liquid should be high as far as possible for removing the Joule heat and for preventing variation in the pH. Where the electrophoretic current is high, as a large Joule heat is generated and the pH variation of the buffer liquid varies greatly, it is necessary to increase the quantity of the buffer liquid circulated through the electrode chamber. In such a case, the electrode chamber is divided into a plurality of sections and independent feed pumps are provided for respective sections. The feed pumps 23 and 24 for circulating the sample liquid may have low speed. The speeds of feed pumps 15 and 16 and 23 and 24 may be the same or different.

In an actual operation of the apparatus, a small quantity of the sample composition which should be transferred to the electrode chamber often remains in the separating chamber so that it takes a long time to perfectly separate the compositions. It is considered that this is caused by the variation in pH of the sample liquid in the separation chamber. The sample liquid in the separation chamber is in contact with the buffer liquid through the dialytic membrane and since the buffer solute can permeate through the membrane, variation in the pH of the sample liquid would not occur. However, after a separation for a long time such variation often occurs. This can be attributable to the variation in the concentration of the amphoteric electrolyte caused by the separation, and the difference in the permeabilites through the membranes of different types of ions. In any case, it is necessary to prevent variation in pH for effective separation. Dialysis is often used for efficiently adjusting the pH of a high molecular weight electrolyte solution so that it is effective to construct the sample liquid tanks 25 and 26 with dialytic membranes and to dip these tanks in the buffer liquid 18 contained in the buffer liquid tank 17 so as to constantly subject to dialysis the sample liquid circulating through the separation chambers 6 and 7.

Fig. 2 shows modified apparatus suitable for carrying out the method of separation of this invention, in which the separation chambers 6 and 7 are partitioned from a sample liquid chamber 29 through dialytic membranes 2a and 2b.

With this apparatus, respective compositions in the sample liquid are separated by migrating to either one of the separating chambers from the central chamber according to their electric charges, whereby high purity separation can be made readily and at a high speed. In this apparatus, where a pH equal to the equipotential point of one substance is taken as an operating condition, only a substance not having charge in the sample liquid and hence does not migrate at the time of current pass is retained in the sample

liquid chamber 29, whereas the other high molecular weight electrolytes are separated into the separation chambers on the opposite sides.

The treating capability of the apparatus can be increased by increasing the migration current for increasing the quantity treated per unit area and unit time, by increasing the dimension of the apparatus, especially the area of the electrophoretic membrane, and by continuously treating the sample.

In order to separate a large quantity of the sample by the continuous operation and by passing only once the sample liquid through the separation chamber, it is necessary to use a large size apparatus and to slow down the inlet speed of the sample liquid so as to elongate the stay time thereof in the separation chamber. This method, however, tends to vary the pH of the sample liquid, thus requiring a certain measure for maintaining the pH at a constant value. Thus, it is advantages to use a number of small apparatus which are connected to dialyze the sample liquid with a buffer liquid.

To improve the concentration effect while separating with a large capacity apparatus it is preferable to make low the inlet speed on the side of increasing the concentration and to make high the inlet speed on the other side so as to suitably determine the liquid quantities passing through respective separation chambers in a unit time.

The electrophoretic membrane and the dialytic membrane utilized in this invention are as follows.

The electrophoretic member should prevent free migration of the solution but passes high molecular weight electrolyte ions and electrolyte ions at the time of electrophoresis. Thus, it does not undergo electro-permeation and must have a certain degree of physical strength. A suitable membrane comprises polyacrylamide gel utilized as a carrier, which is polymerized on a support. Such membrane having a low degree of bridging and a relatively high density is prepared by forming an aqueous solution having a concentration of about 10% (higher than usual) of polyacrylamide monomer incorporated with 1 — 2% (lower than usual value of 3%) of N,N'-methylene bisacryl amide and a polymerization catalyst, applying the aqueous solution onto such support as a filter paper or a nylon mesh, carefully clamping the support between flat sheets such as glass sheets not to entrain air bubbles and then polymerizing. The electrophoretic membrane thus prepared has a high mechanical strength, can maintain well the aqueous solution, and can manifest high migration speed even for amphoteric substances having large molecular weight.

The dialytic membrane does not permeate solution, but permeates electrolyte ions at the time of electrophoresis to manifest electroconductivity. However, it prevents permeation of high molecular weight amphoteric electrolyte and should not permit electric permeation. Moreover, it should have a high physical strength. The dialytic membrane having such characteristics can be prepared by incorporating more than 25 weight % of N,N'-methylene bisacryl amide into polyacrylamide monomer, preparing a concentric aqueous solution of the mixture having a concentration higher than 40%, polymerizing the aqueous solution on a support similar to the electrophoretic membrane to form gel having a high degree of bridging and high density. Since the electric resistance of the membrane tends to increase, it is advantageous to use a support of a small thickness so as to obtain a thin membrane.

CLAIMS

1. A method of continuously separating an amphoteric high molecular weight electrolyte by electrophoresis comprising the steps of adjusting pH of a sample solution containing said amphoteric high molecular weight electrolyte according to an equipotential point of said electrolyte; containing said sample solution in a flowable state with a buffer liquid having the same pH as said sample solution and also in a flowable state via a dialytic membrane; applying electric voltage to said sample solution from the side of said buffer liquid to cause said amphoteric high molecular weight electrolyte in said sample liquid to migrate electrically, thereby separating said amphoteric high molecular weight electrolyte.

2. Apparatus for continuously separating by electrophoresis comprising a casing; an electrophoretic membrane partitioning inside of said casing; dialytic membranes partitioning respective chambers between said electrophoretic membrane and both side walls of said casing; electrodes respectively disposed in the chambers between said dialytic membranes and said side walls; means for circulating buffer liquid contained in a tank through the chambers containing said electrodes; means for circulating a sample solution containing an amphoteric high molecular weight electrolyte and contained in another tank through chambers between said electrophoretic membrane and said dialytic membranes; and means for applying DC voltage across said electrodes.

3. The apparatus according to claim 1 wherein said tank containing said solution is made of a dialytic membrane which is dipped in said buffer liquid.

4. The apparatus according to claim 1 wherein a chamber between said dialytic membranes is partitioned by two spaced apart electrophoretic membranes.

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